

OFFICE OF NAVAL RESEARCH  
FINAL REPORT  
PUBLICATIONS/PATENTS/PRESENTATIONS/HONORS/STUDENTS REPORT

for

GRANT or CONTRACT: N00014-97-1-0634

PR Number 98PR02788-00

CATECHOLAMINE SECRETION FROM INDIVIDUAL CELLS

R. Mark Wightman, Principal Investigator

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July 20, 1998

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PART I

OFFICE OF NAVAL RESEARCH  
PUBLICATIONS/PATENTS/PRESENTATIONS/HONORS REPORT

PR Number: 98PR02788-00

Contract/Grant Number: N00014-97-1-0634

Contract/Grant Title: Catecholamine Secretion from Individual Cells

Principal Investigator: R. Mark Wightman

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- a. Number of papers submitted to refereed journals, but not published: 0
- b. + Number of papers published in refereed journals (for each, provide a complete citation): 2
- c. + Number of books or chapters submitted, but not yet published: 0
- d. + Number of books or chapters published (for each, provide a complete citation): 2
- e. + Number of printed technical reports/non-refereed papers (for each, provide a complete citation): 0
- f. Number of patents filed: 0
- g. + Number of patents granted (for each, provide a complete citation): 0
- h. + Number of invited presentations (for each, provide a complete citation): 16
- i. + Number of submitted presentations (for each, provide a complete citation): 0
- j. + Honors/Awards/Prizes for contract/grant employees (list attached): 1  
(This might include Scientific Society Awards/Offices, Selection as Editors, Promotions, Faculty Awards/Offices, etc.)
- k. Total number of Full-time equivalent Graduate Students and Post-Doctoral associates supported during this period, under this R&T project number: 3
  - Graduate Students: 3
  - Post-Doctoral Associates: 0
  - including the number of,
    - Female Graduate Students: 1
    - Female Post-Doctoral Associates: 0
    - Minority\* Graduate Students: 0
    - Minority\* Post-Doctoral Associates: 0
    - Asian Graduate Students: 0
    - Asian Post-Doctoral Associates: 0
- l. + Other funding (list agency, grant title, amount received this year, total amount, period of performance and a brief statement regarding the relationship of that research to your ONR grant)
- + Use the letter and an appropriate title as a heading for your list, e.g.:

END OF YEAR REPORT: R. Mark Wightman

b. Published papers in refereed journals:

Quantal Corelease of Histamine and 5-Hydroxytryptamine from Mast Cells and the Effects of Prior Incubation. K. Pihel, S. Hsieh, J.W. Jorgenson and R.M. Wightman, *Biochemistry*, 37, 1046-1052 (1998).

Simultaneous Detection of Catecholamine Exocytosis and  $\text{Ca}^{2+}$  Release from Single Bovine Chromaffin Cells Using a Dual Microsensor. Q. Xin and R.M. Wightman, *Anal. Chem.*, 70, 1677-1681 (1998).

d. Published book chapters:

Spatio-Temporal Resolution of Exocytosis from Individual Cells. E. Travis and R.M. Wightman, *Annual Review of Biophysics and Biomolecular Structure*, Vol. 27, 77-103, 1998.

Detection of secretion with electrochemical methods. S. E. Hochstetler, R.M. Wightman, *Biophysics Textbook On-Line*, Victor Bloomfield, editor, accepted May, 1998 (<http://biosci.umn.edu/biophys/OLTB/Textbook.html>).

h. Invited presentations:

"Exploring new domains with microelectrodes" Department of Chemistry, Purdue University, 4/10/97.

"Exploring new domains with microelectrodes" Department of Chemistry, Indiana University, 4/12/97.

"Detection of individual chemical reactions by electrogenerated chemiluminescence" Symposium on Single Molecule Detection, American Chemical Society National Meeting, San Francisco, CA, 4/14/97.

"Exploring new domains with microelectrodes" McElvain Lecturer (graduate student invited speaker), Department of Chemistry, University of Wisconsin, 4/24/97.

"Catecholamine secretion from single cells", Office of Naval Research, Arlington, VA 8/6/97.

"Observation of individual chemical reactions" National Institutes of Health, Bethesda, Md, 8/7/97.

"Chemical analysis in microdomains" Society of Analytical Chemists of Pittsburgh, Pittsburgh, PA, 9/8/97.

"Monitoring neurotransmitters in real time" Department of Chemistry, King's College, London, 9/17/97.

"Monitoring neurotransmitters in real time" Department of Chemistry, University of Abo, Finland, 9/22/97.

"High speed electrogenerated chemiluminescence" Department of Chemistry, University of Padua, Italy, 9/24/97.

"Fast-scan cyclic voltammetry at carbon fiber microelectrodes" Conference on the Electrochemistry of Carbon, Case Western Reserve University, 10/20/97.

"Monitoring neurotransmitters in real time" Symposium on cellular and molecular biodynamics, Rutgers University, Newark, State University, 11/17/97.

"Monitoring neurotransmitters in real time" Pharmacology Department, UNC, 11/97.

"Monitoring neurotransmitters in real time" Werner Lecturer, Department of Chemistry, University of Kansas, 12/8/97.

"Monitoring neurotransmitters in real time" Neurobiology colloquium, UNC, 2/2/98.

"Watching individual chemical reactions in femtoliter volumes" Symposium on Analytical Chemistry in Nanoliter Volumes, Pittsburgh Conference, New Orleans, LA 3/2/98

"Chemical analysis with cyclic voltammetry" Symposium on Electroanalytical Chemistry in Flowing Streams, Pittsburgh Conference, New Orleans, LA 3/2/98

j. Honors/Awards

President, Society for Electroanalytical Chemistry

I. Other Funding: R. Mark Wightman, July 15, 1997

Supporting Agency	Project Title	Costs <sup>a</sup> 1997-1998	Award Amount	Total Period Covered	% of Effort
1. Current Support					
NSF	Microvoltammetric Electrodes (NSF-CHE 9800560)	187,300	487,300	4/98 - 4/01	20%
PHS	Electroanalysis of Neurotransmitters and Modulators (NIH NS 15841)	166,191	659,262	1/97 - 12/00	20%
PHS	Dynamics of <u>In Vivo</u> Dopamine Release	154,070	590,358	3/97 - 2/01	20%
ONR	Catecholamine Secretion from Individual Cells (313v002)	50,000	200,000	4/97 - 1/00	20%
2. Pending Support:					
PHS	Parkinson's Disease Center (NIH NS 38409)	0	841,908	12/98-11/03	20%

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<sup>a</sup> Costs include indirect costs at a 44.5 % rate.

None of the existing grants overlap with the grant from ONR. The grant entitled Dynamics of In Vivo Dopamine Release (NIH DA 10900) concern measurements inside the rat brain. The grant entitled Microvoltammetric Electrodes (NSF-CHE 9800560) deals with the fundamental aspects of these devices. The grant entitled Electroanalysis of Neurotransmitters and Modulators (NIH NS 15841) is concerned with the development of new detection techniques for molecular oxygen, ascorbate, and neurotransmitters. The pending proposal at PHS (Parkinson's Disease Center, (NIH NS 38409)) is a proposal to continue the ONR sponsored research which will terminate this fall.

END OF YEAR REPORT: R. Mark Wightman

PART II.

A. Principal Investigator: R. Mark Wightman

B. Telephone Number: 919-962-1472

C. Cognizant ONR Scientific Officer: Joel Davis, Cognitive and Neural Science and Technology Division

D. Brief description of project:

The dynamic processes of chemical communication at single biological cells will be measured based on recent advances in microanalytical chemistry made in this laboratory. Many cells, including neurons, communicate by secretion of chemical substances by exocytosis where substances are extruded into the extracellular space following fusion of the vesicle and plasma membranes. Ultramicroelectrodes provide sufficient chemical, spatial, and temporal resolution which enable individual exocytotic events to be resolved for cells which secrete catecholamines. Thus, this process can be monitored, manipulated, and understood in a way not previously possible.

E. Significant results during last year.

A major accomplishment of the present year has been the expansion of our technique to the measurement of secretion at mast cells. These cells secrete both histamine and 5-hydroxytryptamine. These substances both serve as chemical messengers in the immune system and are secreted when the mast cell is in the presence of an antigen. Our findings show that both amines are secreted from the same vesicles. However, we find that the storage of these substances in the vesicles is regulated by the other contents of the vesicle. The primary substance is heparin. Its high ionic charge forms a matrix into which the amines are strongly associated. This association prevents rapid exchange of the amines and also limits their rate of secretion. In addition, we developed an amperometric sensor with an attached fluorescent dye. The specific dye employed changes its fluorescence when it complexes calcium ions. This enables both  $\text{Ca}^{2+}$  changes and electroactive amines to be monitored at the surface of a cell. This dual monitoring capability allows new insights into the factors involved in secretion from a variety of cell types.

Overall, the support of ONR in this research has resulted in several "firsts". This research was the first to demonstrate release of substances from single granules. We established for the first time that the rate of release is limited by the rate of dissociation of the vesicle contents. Finally, we made some of the earliest simultaneous measurements of  $\text{Ca}^{2+}$  entry and vesicular release.

G. List of names of graduate students and post-doctorals currently working on the project.

Graduate students. E. Travis, S. Hochstetler, M. Mundorf  
Postdoctorals: No suitable candidate has yet been found.

## End of Year Report, Part III

R. Mark Wightman, P.I.; PR number: 98PR02788-00

### Figure Legends.

1. The cartoon in this figure depicts a single biological cell in culture. The carbon-fiber microelectrode is placed adjacent to the cell to measure secretion. A typical amperometric record of exocytotic release of catecholamines from chromaffin cells isolated from the adrenal gland is shown in the upper part of the cartoon. Also shown are the pipette used to introduce chemical secretory agents onto the cell, and a light beam ( $h\nu$ ) used to excite fluorescence of fura-2, a calcium chelating agent.

2. **Latency for exocytosis following stimulation.** Upper. Application of a chemical stimulus (trace A) does not lead to an immediate generation of exocytotic events (results in B from a single, representative cell). The time-delay to the first vesicle detected by a carbon fiber microelectrode is indicated by the double ended arrow. This was determined at multiple cells and histograms were constructed to compare the latencies found with two different secretagogues. Lower: Histograms that demonstrate differences in the exocytotic triggering by nicotine and potassium. The relatively large number of short onset latencies for nicotine-stimulated cells compared to potassium-stimulated cells appears to indicate highly co-localized nicotinic-sensitive calcium channels close to sites of exocytosis. For the potassium histogram, the decay time constant is 588 ms. ( $n = 85$  stimulations); for nicotine histogram it is 104 ms ( $n = 75$  stimulations).

3. **Cyclic Voltammetric Measurement of Quantal Cosecretion of Histamine and 5-Hydroxytryptamine from a Mast Cell.** A carbon fiber microelectrode was placed adjacent to an isolated mouse peritoneal mast cell. The scan rate was  $800 \text{ V s}^{-1}$  with scans repeated at 33 ms intervals. The color plot shows the response during exocytosis evoked by the  $\text{Ca}^{2+}$  ionophore A23187. Traces for histamine (upper) and 5-hydroxytryptamine (lower) were obtained from the average voltammetric current sampled on successive scans around the respective peak potentials; currents were converted to concentration with calibration curves. The coincident concentration spikes indicate that histamine and 5-hydroxytryptamine are being coreleased from individual secretory vesicles. A cyclic voltammogram from the third secretory spike is shown in the upper left.

# CATECHOLAMINE SECRETION FROM INDIVIDUAL CELLS

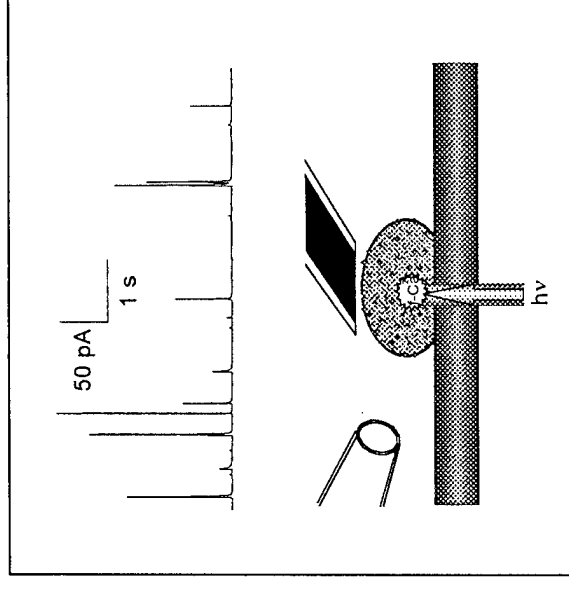
R. Mark Wightman, Department of Chemistry, University of North Carolina at Chapel Hill

## Technology Issues:

Electroanalysis in Microenvironments  
Chemical Analysis at Individual Biological Cells  
Monitor Chemical Secretion from Cells

## Objectives:

Understand the Exocytotic Mechanism of Chemical Secretion  
through Direct, Real-Time Measurements  
Monitor  $\text{Ca}^{2+}$  Entry Coupled with Chemical Secretion



## Approach:

- Microelectrodes placed next to single cells.
- Employ cyclic voltammetry and amperometry for electrochemical analysis
- Add  $\text{Ca}^{2+}$ -responsive fluorescent dye to carbon fiber electrode surface.

## Accomplishments:

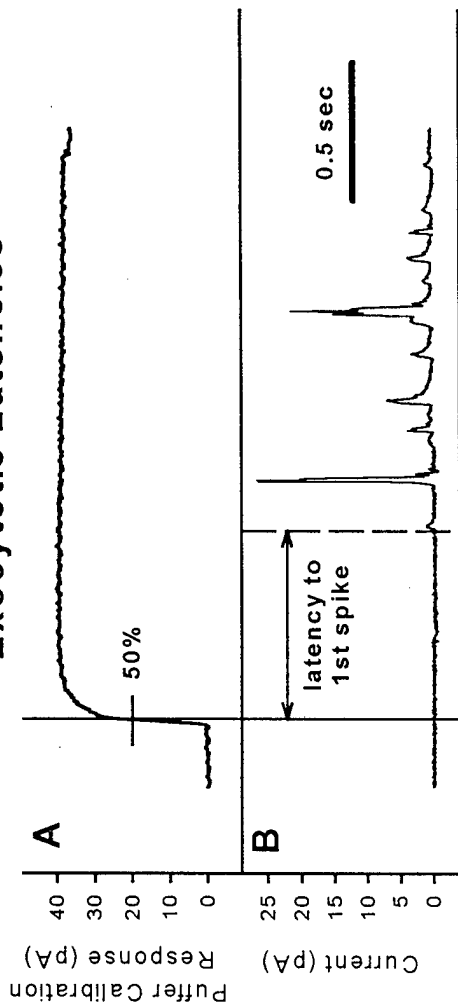
- Measured initial single vesicle secretory events for catecholamines from adrenal cells.
- Measured single vesicle secretory events of histamine and 5-hydroxytryptamine occurring at mast cells (part of the immune response)
- Observed extracellular  $\text{Ca}^{2+}$  via fluorescence and secretion via electrochemistry.

## Impact:

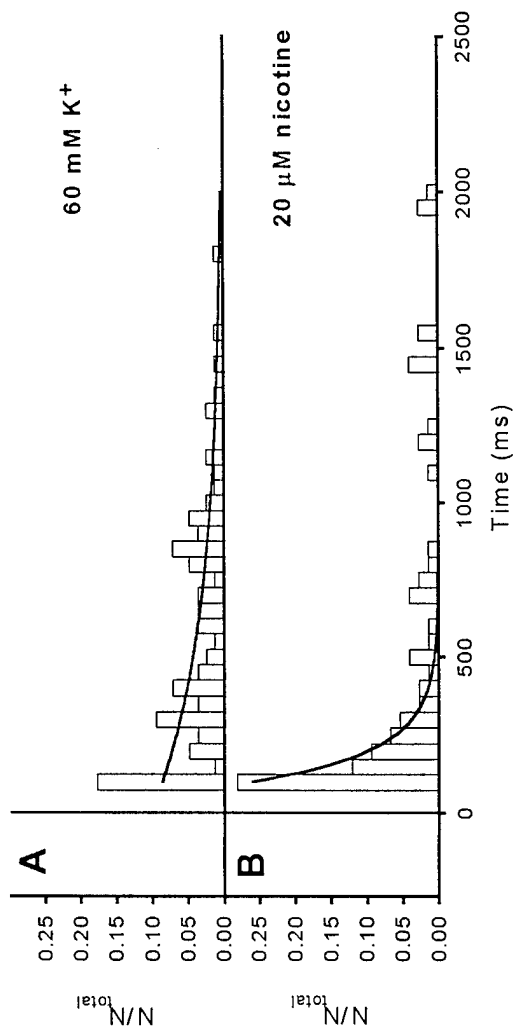
- Established that rate of initial event depends on secretagogue.
- Establish the effects of genetic alterations of the vesicle transporter systems in mast cell vesicles.
- Established that  $\text{Ca}^{2+}$  extrusion occurs simultaneously with exocytosis (Anal. Chem, 70, 1677 (1998)).



# Time-Resolved Stimulus Application and Exocytotic Latencies



## Latency Histograms for 60 mM $K^+$ and 20 $\mu M$ Nicotine



### 3. Simultaneous Electrochemical Measurement of Histamine and 5-HT Coreleased from Individual Secretory Vesicles

